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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/590,705	06/14/2007	Shin-ichi Hashimoto	00005.001301.	8726

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EXAMINER	
MEAH, MOHAMMAD Y	
ART UNIT	PAPER NUMBER
1652	
MAIL DATE	DELIVERY MODE
06/08/2010	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/590,705	HASHIMOTO ET AL.	
	Examiner	Art Unit	
	MD. YOUNUS MEAH	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *03/23/10* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-3 and 5-15* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-3 and 5-15* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Claims 1-3 and 5-15 were examined in the previous action.

Claims 1-3 and 5-15 are currently pending in the instant application.

In response to a previous office action, (mailed on 11//24/2009), applicants on 03/23/2010 amended claims 1-3 and 5-7. Applicants' response on 03/23/10 is acknowledged. Claims 1-3 and 5-15 are under consideration.

Applicants' arguments filed on 03/23/10 have been fully considered but they are found unpersuasive. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Objection

Claim 1 and 6 are objected for comprising non-elected subject matter (SEQ ID NO: 5, 7, 9, 11, 13 and 15). Appropriate correction is required.

Claim 1 is objected for reciting "microorganism expressing a heterologous DNA encoding NADH-dehydrogenase...". It should recite "microorganism expressing a heterologous DNA encoding a NADH-dehydrogenase...." Appropriate correction is required.

Claims 2-3, 5 are objected for reciting "wherein the heterologous DNA encoding NADH dehydrogenase (i) or (ii)". It should recite "wherein the heterologous DNA of (i) or (ii)" .Otherwise as recited, it appears that (i) and (ii) are describing the NADH dehydrogenase. Appropriate correction is required.

Claim 6 is objected for reciting "wherein the NADH dehydrogenase is ---a polypeptide having an amino acid sequence selected from.. SEQ ID NO: 4-----.". It should recite "wherein the NADH dehydrogenase is (1) a polypeptide having an amino acid sequence selected from.. SEQ ID NO: 4...and 16, or (2) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4....and 16 wherein 1 to 20 amino acids.....". Appropriate correction is required.

Claims 5 and 7 are objected for reciting "within the plasmid pCS-CGndh within *Escherichia coli* DH5α./pCS-CGndh". It should recite "within the plasmid pCS-CGndh, and wherein the microorganism is *Escherichia coli* DH5α./pCS-CGndh" Appropriate correction is required.

Claim 7 is objected to under 37 CFR 1.75 as being a substantial duplicate of claim 3. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k).

35 U.S.C 112 1st Paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-3 and 5-15 remain rejected under 35 U.S.C. 112, first paragraph, as

containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 1-3 and 5-15 are directed to a method of production of amino acids by using a microorganism expressing a heterologous polynucleotide which is approximately 69.8% sequence identical to the polynucleotide of SEQ ID NO: 3 encoding a protein having NADH dehydrogenase activity or any heterologous DNA encoding NADH dehydrogenase wherein said DNA hybridizes to a DNA having any fragment which is complementary to SEQ ID NO: 3. Claim 1 recites "which hybridizes under stringent conditions with a DNA having a nucleotide sequence complementary to the nucleotide sequence of a DNA of SEQ ID NO: 3". The term "a nucleotide sequence complementary" in its broadest reasonable interpretation is read as a fragment of any size which is complementary to the DNA of SEQ ID NO: 3 recited.

A calculation of the Tm of the polynucleotide recited in claim 1 shows that under the hybridization conditions recited, the recited polynucleotides can be approximately 69.8% sequence identical to the polynucleotide of SEQ ID NO: 3. Using the well known equation of Meinkoth and Wahl (Current Protocols in Molecular Biology, Hybridization Analysis of DNA Blots, pages 2.10.8-2.10.11, 1993), Tm = 81.5 ºC $+16.6x\log_{10}[Na+]$ +0.41x(%GC) - .61x(%form) – 500/L, the corresponding Tm for the polynucleotide recited is approximately 95.2ºC assuming a G+C content of 50% and neglecting the term 500/L, where L is the length in nucleotides of the probe (95.2 ºC = 81.5 + $16.6x\log_{10}[3.9x2/20]$ +0.41x(%50) - .61(%form = 0); for 2xSSC the molar

concentration of Na+ is 3.9). As known in the art, Tm is reduced by approximately 1 ºC for each 1% mismatching, therefore under the conditions recited (2xSSC and 65 ºC), a wash at 65 ºC is equivalent to approximately 30.2% mismatching (30.2% =95.2ºC – 65ºC). This level of mismatching amounts to 424 nucleotides which can be modified (424 = 0.302x1404) within SEQ ID NO: 3 (1404 nucleotides). Thus, the genus of polynucleotides recited encompass polynucleotides having at least 69.8% sequence identity with the polynucleotide of SEQ ID NO: 3. Since each of these nucleotide mismatches can affect a codon, the genus of polynucleotides recited can encompass polynucleotides where a great majority of the mismatches affects a codon, thus these polynucleotides can encompass polynucleotides having a protein having essentially no structural similarity with the polypeptide of SEQ ID NO: 4.

As discussed in the written description guidelines the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. A representative number of species means that the species, which are adequately described, are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus. The specification teaches method of production

of amino acid by using a microorganism expressing only a few heterologous DNA encoding NADH dehydrogenase of SEQ ID NO: 4. Moreover, the specification fails to describe any other representative species by sufficient identifying characteristics or properties to show that applicant was in possession of the claimed genus.

There is no structure-function correlation with regard to the members of the genus of polypeptides having NADH dehydrogenase activity encoded by the polynucleotides recited in the instant claims. The specification discloses the structure of a few polynucleotides encoding a polypeptide having NADH dehydrogenase activity. No disclosure of the structural features required in any polynucleotide encoding the recited proteins has been provided. Nothing is known with regard to the structural elements that are essential and those that can be modified in the polynucleotide of SEQ ID NO: 3 such that one could make a variant that encodes a protein having the desired activity. However the claimed method requires a genus of polynucleotides encoding a genus of polypeptides having essentially any structure (as for example any number of amino acid variations of SEQ ID NO: 4; encoded by polynucleotides having at least 69.8% sequence identity to the polynucleotide of SEQ ID NO: 3). This includes various allelic variants of the polypeptide of SEQ ID NO: 4 that the specification does not disclose. Therefore one of skill in the art would not recognize from the disclosure that applicants' were in possession of the claimed invention.

Applicants' are referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov.

Claims 1-3, 5-15 remain rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of production of amino acids by using a microorganism expressing heterologous DNAs comprising SEQ ID NOs: 3 which encode the NADH dehydrogenase of SEQ ID NO: 4, does not reasonably provide enablement for a method of production of amino acids by using a microorganism expressing a heterologous polynucleotide which is approximately 69.8% sequence identical to the polynucleotide of SEQ ID NO: 3 encoding a protein having NADH dehydrogenase activity or any heterologous DNA encoding a NADH dehydrogenase wherein said DNA hybridizes to a DNA having any fragment which is complementary to the polynucleotides of SEQ ID NO: 3. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

According to MPEP 2164.01(a), factors considered when determining whether there is sufficient evidence to support a determination that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is "undue" include, but are not limited to: (A) The breadth of the claims; (B) The nature of the invention; (C) The state of the prior art; (D) The level of one of ordinary skill; (E) The level of predictability in the art; (F) The amount of direction provided by the inventor; (G) The existence of working examples; and (H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure.

MPEP§ 2164.04 states that while the analysis and conclusion of a lack of enablement are based on the factors discussed in MPEP § 2164.01(a) and the evidence as a whole, it is not necessary to discuss each factor in the written enablement rejection. The language should focus on those factors, reasons, and evidence that lead the examiner to conclude that the specification fails to teach how to make and use the claimed invention without undue experimentation, or that the scope of any enablement provided to one skilled in the art is not commensurate with the scope of protection sought by the claims. Accordingly, the factors most relevant to the instant rejection are addressed in detail below.

Claims 1-3, 5-15 are so broad as to encompass a method of production of amino acids by using a microorganism expressing a heterologous polynucleotide which is approximately 69.8% sequence identical to the polynucleotide of SEQ ID NO: 3 encoding a protein having NADH dehydrogenase activity or any heterologous DNA encoding any NADH dehydrogenase wherein said DNA hybridizes to a DNA having any fragment which is complementary to SEQ ID NO: 3. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of polynucleotides of unknown structure encompassed by the claims. Since the amino acid sequence of a protein encoded by a given polynucleotide determines its structural and functional properties, predictability of which changes can be tolerated in the encoded protein's amino acid sequence to obtain the desired activity requires knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly

intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. However, in this case the disclosure is limited to a few polynucleotides encoding NADH dehydrogenases, including one comprising SEQ ID NO: 3 that encodes the polypeptide of SEQ ID NOs: 4. It would require undue experimentation of the skilled artisan to make and use the claimed polynucleotides encoding said polypeptides. The specification provides no guidance with regard to the making of variants and mutants or with regard to other uses. In view of the great breadth of the claims, amount of experimentation required to make the claimed polypeptides, the lack of guidance, working examples, and unpredictability of the art in predicting function from a polypeptide primary structure, the claimed invention would require undue experimentation. As such, the specification fails to teach one of ordinary skill how to make and/or use the polynucleotides encompassed by the claims.

While isolation techniques, recombinant and mutagenesis techniques are known, and it is routine in the art to screen for multiple substitutions or multiple modifications as encompassed by the instant claims, the specific amino acid positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims because the specification does not establish: (A) regions in the polynucleotide (SEQ ID NO: 3)

structure which may be modified without affecting its original activity of encoding a NADH dehydrogenase; (B) the general tolerance of NADH dehydrogenases to modification and extent of such tolerance; (C) a rational and predictable scheme for modifying any nucleotide with an expectation of obtaining a protein having the desired biological function; and (D) sufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including polynucleotides with an enormous number of nucleotide modifications to SEQ ID NO: 3. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of polynucleotides having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Argument

Applicants' arguments against the rejection of claims 1-3, 5-15 under 35 U.S.C. 112, first paragraph are not found persuasive. Applicants argue that whole DNA is not required as a hybridization probe, 15-20 nucleotides are enough. Applicants by citing an example illustrate that 21 continuous nucleotide of 30 nucleotide of a test DNA will hybridize with a test sample. Applicants' argument is considered but found unpersuasive. Applicants' argument about the probe is acknowledged however the

claims are directed to a process which requires DNAs that encode a NADH dehydrogenase, not probes. As explained above, the recited hybridization conditions of claim 1 result in a 30% variation of the polynucleotide molecule of SEQ ID NO: 3 anywhere within the polynucleotide. As indicated above, a polynucleotide having 30% variation within SEQ ID NO: 3 can encompass a polynucleotide which encodes essentially any protein having NADH dehydrogenase activity which has very little structural similarity with the polypeptide of SEQ ID NO: 4 or up to 140 amino acids that can be altered in any way (140= 30%x468).

The reference of Witkowski et al. (Biochemistry. 1999 Sep 7; 38(36): 11643-50) teaches that only a single amino acid substitution results in conversion of the activity of a polypeptide to a second, distinct activity (see e.g., Table 1, page 11647). Therefore one of skill in the art will need to do enormous number of experimentation to find out the coding sequence of the polynucleotide. This would clearly constitute **undue** experimentation. While enablement is not precluded by the necessity for routine screening, if a large amount of screening is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. Such guidance has **not** been provided in the instant specification.

Claim Rejections 35 U.S.C 103a

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all Obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the

subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-3, 6, 8-10, 14 and 15 remain rejected under 35 U.S.C. 103(a) by Bott *et al* (*J. Biotechnol*, 2003, 129-153, from IDS) in view of Molenaar *et al* (J. *Bactorl*, 2000, 6884-6891, from IDS), Hollander *et al* (*Appl Micriobiol Biotechnol* 1994, 42, 508-515) and Nakagawa *et al* (US20020197605) for reasons of record. This rejection was discussed at length in the previous office action and discussed it again.

Bott *et al* describes the production of amino acids, such as, glutamate and L-lysine (page 130 left column, 1st pargh) by *Corynebacterium glutamicum* and that respiratory chain enzymes involved in the oxidative phosphorylation in the aerobic respiration of *Corynebacterium glutamicum* are useful in amino-acid production and one such enzyme is NADH dehydrogenase (abstract and FIG 1). However; Bott *et al* do not teach the method of producing amino acid by using microorganism transformed with heterologous NADH dehydrogenase derived from *Corynebacterium glutamicum*.

Molenaar *et al* teach NADH dehydrogenase gene of SEQ ID NO: 1 encoding NADH dehydrogenase (100% identical to applicants SEQ ID NO: 4) isolated from *Corynebacterium glutamicum* which is 100% identical to applicant NADH dehydrogenase gene of SEQ ID NO: 3 and Molenaar *et al* teach said NADH dehydrogenase is Type II NADH, wherein in the reaction number of proton discharged per electron is zero (page 6884, right column last paragraph). Molenaar *et al.* also teach

that *Corynebacterium glutamicum* only expresses type II NADH dehydrogenase (page 6887, right column 1st paragraph).

Methods of expressing endogenous and exogenous genes in a host cell are well known in art to enhance the production of proteins and small organic compound. For example, Nakagawa *et al* teach an improved production of fine chemicals, such as, amino acid, and vitamins, by using a production strain of transformed host cell such as *E. coli* (subject matter of claims 8-9) with exogenous gene encoding desired enzymatic activities (page 7 paragraphs 0179-0191; page 14, paragraphs 0312-0313). Amino acids such as, L-lysine is industrially important chemicals.

It is well known in art that NADH is produced in several reactions in the amino acid biosynthesis pathway of *Corynebacterium glutamicum* (Hollander *et al. Appl Micriobiol Biotechnol* 1994, 42, 508-515, Fig 1 at page 509). NADH dehydrogenase converts NADH to NAD. Hollander *et al* teach that quantitative yield of lysine can be produced from glucose in a fermentation system comprising *Corynebacterium,* if NADH and NADPH are consumed (its concentration is decreased) (last paragraph, page 514). Therefore, since type-II membrane bound NADH dehydrogenase of *Corynebacterium glutamicum* converts NADH to NAD, by doing so it depletes the NADH and increase the production of lysine from glucose. Although *E. coli* comprises two NADPH dehydrogenases (Type 1 and type II) they are different from that of NADH dehydrogenase (100% identical to applicants SEQ ID NO: 4) isolated from *Corynebacterium glutamicum* (only 27% sequence homology, page 132, Bott et al.). Therefore, in order to produce amino acid in large scale, one of ordinary skill in the art is

motivated to express *E. coli* (as taught Nakagawa *et al*) with NADH dehydrogenase gene of SEQ ID NO: 1 of *Corynebacterium glutamicum* of Molenaar *et al* and use the said transformed microorganism in the method of production of amino acid.

As such it would have been obvious to one of ordinary skill in the art to use Molenaar *et al* NADH gene of SEQ ID NO: 1 encoding a type II NADH dehydrogenase (which discharge zero proton per electron) isolated from *Corynebacterium glutamicum* which is 100% identical to applicant NADH gene of SEQ ID NO: 3 express the said gene in *E. coli* and use the transformed E. *coli* to the method of production of amino acid.

Claims 11-13 are rejected under 35 U.S.C. 103(a) by Bott *et al* (*J. Biotechnol*, 2003, 129-153, from IDS) in view of Molenaar *et al* (J. *Bactorl*, 2000, 6884-6891), Hollander *et al* (*Appl Micriobiol. Biotechnol.* 1994, 42, 508-515) and Nakagawa *et al* (US20020197605).

The teaching of Bott *et al*, Hollander *et al* and Nakagawa *et al* is discussed above for the 35 U.S.C. 103(a) rejection of claims 1-3, 6, 8-9, 14-15. However Bott *et al*, Hollander *et al* and Nakagawa *et al* do not teach explicitly a process of producing amino acids using *Corynebacterium glutamicum* expressing heterologous NADH-II dehydrogenase gene of SEQ ID NO: 1.

Since Bott *et al* describes the production of amino acids by *Corynebacterium glutamicum* in the biosynthesis of amino acids use different respiratory chain enzymes and one of the enzymes used is NADH dehydrogenase (NADH-II), in order to further

enhance the production of amino acids by *Corynebacterium*, one ordinary skill in the art is motivated to express heterologous NADH-II dehydrogenase gene of SEQ ID NO: 1 of Molenaar *et al.*) in *Corynebacterium or Corynebacterium glutamicum*. One of ordinary skill in the art would reasonably expect this to increase the amount of the NADH-II dehydrogenase produced in the *Corynebacerium* and therefore, enhance the amino acid production.

As such it would have been obvious to one of ordinary skill in the art to use Molenaar *et al* NADH dehydrogenase gene of SEQ ID NO: 1 encoding type II NADH dehydrogenase isolated from *Corynebacterium glutamicum* which is 100% identical to applicant NADH gene of SEQ ID NO: 3 express the said gene in *corynebacterium or Corynebacterium glutamicum* and use the transformed *Corynebacterium* or *Corynebacterium glutamicum* to the method of production of amino acid, as taught by Bott *et al*.

Arguments and response

Applicants argue, at pages 8-9 of their amendment of 03/23/10, that one of ordinary skill in the art would not combine the recited prior arts in the 35 USC 103 rejection above because Nakai teaches away *E. coli* NADH-II in amino acid production in *E. coli*. Applicants also argue that *C. glutamicum* has 2 of the same kinds of NADH dehydrogenases found in *E. coli*. Applicants' arguments have been fully considered, but they found unpersuasive. In fact, as explained in the 103(a) rejection above Bott et al and Molenaar *et al* teach that *C. glutamicum* comprises only one

NADH dehydrogenase which is membrane bound NADH-II type whereas *E. coli* comprises both NADH-I and NADH-II. As argued in the previous rejection, *E. coli* comprises two NADPH dehydrogenases (Type I and type II) they are different from NADH II dehydrogenase (100% identical to applicants SEQ ID NO: 4) isolated from *Corynebacterium glutamicum* (only 27% sequence homology to *E. coli* NADH-II, page 132, Bott et al.). As discussed above *Corynebacterium glutamicum* is an amino acid producing microorganism having only a type II NADH dehydrogenase. Said NADH dehydrogenase involved as a primary dehydrogenase, linked with central metabolism, in the respiratory chain of *Corynebacterium glutamicum* and its growth and for the production of amino acids. One of ordinary skill in the art would recognize that NADH-II dehydrogenase of *Corynebacterium glutamicum* is involved in amino acid production in *Corynebacterium glutamicum*. With regard to the teachings of Nakai *et al* (US2002/0160461), it is noted that (1) NADH-II dehydrogenase (100% identical to applicants SEQ ID NO: 4) isolated from *Corynebacterium glutamicum* (only 27% sequence homology to that of *E. coli)* is not the same NADH-II of *E. coli;* and (2) Nakai et al teach that (Table 2, page 20) over-expression of cytochrome [bd-type] oxidoreductase (CYO) in *E. coli* increases the amino acid production. Production of lysine in transformed *E .coli* strain increases from .29g/L (wild-type) to .48-.53 g/L (over-expressed CYO). NADH-I is involved in cytochrome bo-type oxidoreductase pathway, not NADH-II (pages 1-2, Nakai et al). In Nakai *et al's*, *E. coli* strain over-expressed with cytochrome [bd-type] oxidoreductase (CYO) uses NADH I dehydrogenase in the amino acid production, not the NADH-II. Therefore a mutation of

NADH-II in the said *E. coli* strain does shows little effect on amino acid production. The CYO over-expressed *E. coli* strain produces 0.48 g/L of lysine when NADH-II of said strain is not mutated and produces 0.53 g/L of lysine when NADH-II is mutated (within experimental error 0.48 and 0.53 are same, Table 2, page 20). Applicants' argument that Corynebacterium *glutamicum* probably uses other dehydrogenases that consume NADH/NADPH in the production of amino acid is considered. However, as explained above since *Corynebacterium glutamicum* uses type-II NADH dehydrogenase in the central metabolism, in the respiratory chain, growth and amino acid biosynthesis, one ordinary skill in the art would introduce Molenaar *et al* NADH gene of SEQ ID NO: 1 isolated from *Corynebacterium glutamicum* which is 100% identical to applicant NADH gene of SEQ ID NO: 3 to a microorganism to enhance the production of amino acid. Moreover as explained above NADH dehydrogenase converts NADH to NAD. Hollander *et al* teach that quantitative yield of lysine can be produced from glucose in a fermentation system comprising *Corynebacterium,* if NADH and NADPH are consumed (its concentration is decreased) (last paragraph, page 514). Therefore, since type-II membrane bound NADH dehydrogenase of *Corynebacterium glutamicum* converts NADH to NAD, by doing so it depletes the NADH and increase the production of lysine from glucose. Therefore, in order to produce amino acid in large scale, one of ordinary skill in the art is **motivated** to express *E. coli* (as taught Nakagawa *et al*) with NADH dehydrogenase gene of SEQ ID NO: 1 (encoding Type-II membrane bound NADH dehydrogenase) of *Corynebacterium glutamicum* of Molenaar *et al* and use the said

transformed microorganism in the method of production of amino acid. Thus, the claimed invention remains *prima facie* obvious over the prior art of record.

Allowable Subject Matter/Conclusion

None of the claims are allowable.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Mohammad Meah whose telephone number is 571-272-1261. The examiner can normally be reached on 8:30-5PM.
If attempts to reach the examiner by telephone are unsuccessful, the examiner's Andrew Wang can be reached on 571-272-0811. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Mohammad Younus Meah
Examiner, Art Unit 1652

/Delia M. Ramirez/
Primary Examiner, Art Unit 1652